

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
NATIONAL VETERINARY SERVICES LABORATORIES
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SAM - 104

Parainfluenza-3
V-59
Standard Requirement

Revised July 29, 1983
Supersedes April 19, 1971

Vaccine
Agent

SUPPLEMENTAL ASSAY METHOD

FOR

ISOLATION OF PARAINFLUENZA-3 VIRUS

FROM NASAL SECRETIONS

A. SUMMARY

This method employs a cell culture system for the detection of Parainfluenza-3 virus in nasal secretions taken from calves with cotton-tipped applicators.

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B. MATERIALS AND MEDIA

1. **Cell Cultures.** Multiple 24-well disposable plates (16 x 150 mm) are seeded with primary bovine embryonic kidney (BEK) cells (2nd through 5th passage), free from extraneous agents, at a cell count that will produce a monolayer after 1 day of incubation.

a. Growth Medium

The cells are grown in Minimum Essential Medium (MEM) with additives (Appendix 1) at a temperature of 35 to 37 C in an incubator containing an atmosphere of 5% carbon dioxide (CO₂) and a relative humidity of 70 to 80%. Growth medium is not changed unless excess acidity occurs or cells are not growing well.

b. Diluent

Maintenance medium (Appendix 2) without serum is used to make dilutions.

2. Guinea Pig Erythrocytes for the Hemadsorption (HA) Test.

a. Blood from healthy guinea pigs is collected aseptically in an equal amount of sterile Alsever's solution (Appendix 3).

b. The erythrocytes are washed 3 times in Alsever's solution, and sedimented each time by centrifugation at 1,000 rpm for 15 minutes. Cells are stored at 5 C as a 50% suspension in Alsever's solution.

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c. For the HA test, cells are diluted to a 0.5% suspension in phosphate buffered saline (PBS) (Appendix 4).

C. METHOD

1. Nasal secretion specimens are taken from each animal by inserting a sterile cotton-tipped applicator stick several inches into the nasal passage. A separate applicator is used for each nasal passage. The swabs are immediately immersed in 3 ml of brain-heart infusion broth (with antibiotics) contained in a small plastic tube, frozen, and stored at -70 F until ready for culturing.

2. The fluids containing the swabs are thawed. The fluid is expressed from the cotton by rotating and pressing the applicators against the tube wall. The suspension is then centrifuged at 2,000 rpm for 20 minutes. Two-tenths (0.2) ml amounts of the supernatant are inoculated into each of 5 wells containing the BEK cells. The plates are incubated in a CO₂ incubator at 36 to 37 C for 5 days.

3. A National Veterinary Services Laboratories (NVSL) reference PI-3 virus is used as a positive control for the cell system. A vial is thawed, mixed, and diluted in a maintenance medium without serum (Appendix 2) to a dilution 1 or 2 logs lower than the known 50% endpoint. Five wells are inoculated with 0.2 ml of virus and incubated along with the cells inoculated with the nasal secretion specimen.

4. Negative controls, consisting of 5 uninoculated cell culture wells, are incubated with the test.

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5. The inoculated cell culture tubes are observed daily for cytopathic effect (CPE) characteristic of PI-3 virus. Fluids from tubes showing no CPE at 5 days are passaged, incubated 5 days, and observed for typical CPE.

6. An HA test is performed on all second passage wells which show no CPE. At least one well from each set of 5 per specimen showing CPE is also tested by HA. If one or more tubes in a set show HA, the test is considered positive for PI-3 virus isolation.

a. The HA test method is as follows:

- (1) Fluids are poured from the plates.
- (2) The cells are washed once with PBS (Appendix 4).
- (3) One ml of a 0.5% suspension of guinea pig erythrocytes (RBC) is added to each well.
- (4) The plates are placed so that the cell monolayer is covered with the red cell suspension and allowed to stand 15 to 20 minutes at room temperature.
- (5) The suspension of RBC is poured off and the monolayers are washed 3 times with PBS (Appendix 4).
- (6) The PBS is poured from the plates and the monolayer is examined microscopically for hemadsorption.

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APPENDIX

1. Growth Medium

Edamin	0.5 %
MEM (Eagle with Earles' Salts) q.s. ad	100.0 %
Antibiotics - Penicillin	100.0 units/ml
Streptomycin	100.0 mcg/ml
Gentamicin	50.0 mcg/ml
Amphotericin B	2.5 mcg/ml
Add 10% fetal calf serum	
L-Glutamine	1.0 %

2. Maintenance Medium

Edamin	0.5 %
MEM (Eagle with Earles' salts) q.s. ad	100.0 %
Antibiotics - Penicillin	100.0 units/ml
Streptomycin	100.0 mcg/ml
Gentamicin	50.0 mcg/ml
Amphotericin B	2.5 mcg/ml
L-Glutamine	1.0 %

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3. Alsever's Solution

Dextrose	2.05 %
Sodium citrate	0.8 %
Sodium chloride	0.42 %
Citric acid	0.055%
Distilled H ₂ O q.s. ad	100.0 %

4. Phosphate Buffered Saline (PBS-Dulbecco)

NaCl	0.8 %
KCl	0.02 %
Na ₂ HPO ₄	0.115%
KH ₂ PO ₄	0.02 %
CaCl ₂ (anhy.)	0.01 %
MgCl ₂ 6H ₂ O	0.01 %
Distilled H ₂ O q.s. ad	100.0 %